INTERNATIONAL JOURNAL OF Experimental Pathology

Int. J. Exp. Path. (2012), 93, 18–23

ORIGINAL ARTICLE

New insights in to the treatment of myocardial infarction

Petr Sarapultsev*, Oleg Chupakhin†, Alex Sarapultsev*, Maxim Rantsev‡, Larisa Sidorova§, Svetlana Medvedeva* and Irina Danilova*

**Institute of Immunology and Physiology (IIP) of the Ural Division of Russian Academy of Sciences, †The I. J. Postovsky Institute of Organic Synthesis of the Ural Branch of the RAS, Laboratory of Heterocyclic Compounds, ‡GOU VPO The Ural State Medical Academy and §Federal State Autonomous Educational Institution of Higher Professional Education, Ural Federal University named after the first President of Russia B. N. Yeltsin*

INTERNATIONAL JOURNAL OF EXPERIMENTAL PATHOLOGY

doi: 10.1111/j.1365-2613.2011.00794.x

Received for publication: 19 June 2011
Accepted for publication: 27 September 2011

Correpondence:
Alex Sarapultsev
620219, 106 Pervomayskaya st.,
Ekaterinburg
Tel.: 007 343 3740070
Fax: 007 343 3740070
E-mail: Sarapultsev@sky.ru

Summary

This study investigated the effects of the L-17 compound of the group of substituted 5R1, 6H2-1,3,4-thiadiazine-2-amines on the inflammatory cellular infiltration and myocardial remodelling which occurs after acute myocardial infarction (MI) in rats. The study is based upon recent clinical and experimental work which demonstrated the role of local and systemic inflammatory reactions in postinfarction remodelling. Acute MI in rats was induced by left coronary artery coagulation. Animals were sacrificed on day one, five and seven after MI induction. The myocardiumal samples were taken from all parts of the heart and examined by histology. This included areas of infarction, infraction and areas that were peri-infarctiom and left ventricular areas distant from the damaged tissues. Serum activity of creatine phosphokinase (CPK), aspartate aminotransferase (AST), isoenzymes 1 and 2 and lactate dehydrogenase (LDH1-2) were investigated on the same three days, before and in the process of MI development was investigated (at days 1, 5 and 7). The L-17 compound to not only decreased the area of initial infarction but also changed the pattern of inflammatory reaction in the affected myocardium fundamentally. Laboratory studies of effects of L-17 compound on the development and course of experimental MI showed that administration decreased blood AST and CPK levels significantly and provided useful the data about the correlation between the activity of these enzymes and the dimensions of the significantly necrotic area. In this model of experimental MI the use of the L-17 compound induced led to the replacement of the exudative destructive inflammation that is seen under standard conditions with a more cellular ''productive'' pattern of inflammation, with associated reduction in initial necrosis area and the, decrease in myocardial ischaemia and reperfusion injury may account for the accelerated repair process.

Keywords
inflammation, L-17 compound, myocardial infarction

Advances in cardiology patient care has lead to a decrease in hospital mortality rates among patients with acute myocardial infarction (AMI). However, the continued the persistently high worldwide incidence of ischaemic heart disease supports interest in the pathogenesis and natural history of myocardial damage (Drapkina *et al.* 2000; Arzamastsev *et al.* 2003; Saprunova *et al.* 2003; Golikov, 2004) as this may help in the development of new more effective forms of treatment.

Presently, treatment for ischaemic heart damage relies on the simulati on actions of natural antistressor and anti-ischaemic mechanisms. The administration of metabolites, or of their synthetic analogues, that affect different parts of the pathogenetic chain (Meyerson 1984).

Thus, according to the Guidelines of the Task Force of the European Society of Cardiology (2000) and subsequent Russian Guidelines (2001), treatment for developed MI should rely on the use of beta-blockers, nitrates, calcium

channel blockers and antiplatelet agents (aspirin, thienopyridines, platelet glycoprotein IIb/IIIa receptor antagonists). However, these medications do not affect either the tissue or the systemic the tissue inflammatory process associated with MI, (Smith *et al.* 1977).

The inflammatory reaction is a component not only of the ischaemia but also of the reperfusion injury to the myocardium, thus contributing to the spread of the necrotic area (Tommasi *et al.* 1999). In addition, the pattern of necrosis formation can lead to cause abnormal left ventricular remodelling in MI (Chukaeva *et al.* 2007). Moreover, reduction in scar size is associated with prevention of secondary waves of myocardial necrosis (Sarkisov, 1979).

A number of recent studies have supported this view. Activation of cellular effectors of inflammation has an adverse effect on myocardial function in AMI (Engler *et al.* 1986; Kuzuya *et al.* 1991). The dimensions of the infarction area correlate with indicators of acute-phase response (Myagkov *et al.* 1993). Therefore, lower baseline CRP level are associated with a more favourable prognosis for six months after MI (Hudson *et al.* 1999). The role of the inflammatory reaction in MI is further confirmed by the fact that removing neutrophils from blood or adding agents inhibiting leucocyte infiltration contributed to reducing the dimensions of infarction area in animal studies (Engler *et al.* 1986; Dinerman *et al.* 1990). Therefore the objective of the present study was to investigate the effects of L-17 compound of the group of substituted 5R1, 6H2-1,3,4-thiadiazine-2-amines, known to have effects on the myocardium, on the inflammatory cellular infiltration and myocardial remodelling after acute MI in rats.

Materials and methods

L-17 compound of the group of substituted 5R1, 6H2-1,3,4-thiadiazine-2-amines was used to treat experimental MI in rats (Figure 1).

This compound was synthesized in the Institute of Organic Synthesis, Ural Branch of RAS, as part of a series of several active substances that affect metabolism and inflammation. The compound is a registered invention (United States Patent No 6313111 of 6 November 2001, PCT RF Patent No 2259371 of 27 August 2005). Choice of this compound was because compound L-17 is known to be biologically active and known to have myorelaxing (United States Patent No 4309426 of 20 November 1980), antispasmodic and antiaggregatory (Novikova *et al.* 1992; Logvinova *et al.* 2010) activity but has not yet been investigated in an in vivo pathogenetic model system. The findings in treatment for experimental MI were also protected by a patent (PCT RF Patent No 2395850 of 27 July 2010).

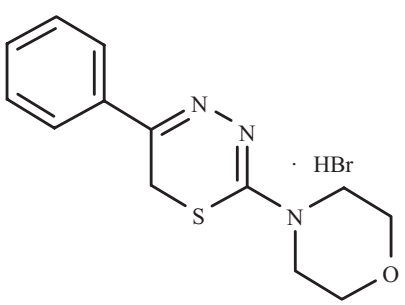

Figure 1 Structural formula of L-17 compound of the group of substituted 5R1, 6H2-1,3,4-thiadiazine-2-amines.

Model of acute MI

Modelling of an AMI in rats was performed as described previously (PCT RF Patent No 2407062 of 20 December 2010). This method is based on operative modelling of MI in rats, which is characterized by a sequence of the following steps: skin is incised and muscles exposed on the left side of the chest; pectoral muscles are spread to expose costal arches and intercostal muscles; the intercostal muscles are dissected at the level of the 4–5 intercostal space for 1 cm; heart is visualized; and coagulation is performed over a standard limited area of the branches of the left coronary artery in its middle or lower third. Coagulation is performed with an 'L'-shaped instrument to give a cauterizing surface area of 2.5×3.0 mm, preheated using an alcohol lamp which allows clear visualization of the heart during coagulation. The thoracotomy wound is closed with a running suture using an atraumatic needle; pneumothorax is eliminated using a needle syringe; and the skin incision is closed. This method increases the likelihood of a positive result (i.e an AMI) while minimizing operative time and increasing the time for the experiment itself. In addition this method neither disturbs the heart rhythm nor causes arrhythmias associated with an electric current effect.

Within 10 min after infarction modelling, animals' behavioural reactions return fully to the preoperative level. No deaths were recorded. Assessment of the animals' behaviour at 24 h revealed no signs of abnormal behavioural reactions: the animals were active; and they react to sound and light stimulation without any delay.

Animal preparation

Twelve-week-old male random-bred male rats were used. They were housed at 23–25 °C in a 12:12-h light–dark cycle with *ad libitum* food and water.

Acute MI was induced by left coronary artery coagulation as described previously. Rats were anesthetized by intraperitoneal injection of ketamine (50 mg/kg) and xylazine (10 mg/kg). They were intubated and ventilated, and the procedure was performed as outlined above. Coagulation was performed over the standard limited area of the branches of the left coronary artery in its middle or lower third. Controls were sham-operated rats which underwent the same operation but, without controls were coronary artery coagulation. The animal experiments were approved and performed according to the Direction 86/609/EEC (1986) of the EU Council and the European convention on the Protection of Vertebrates used for Experimental and

Other Scientific Purposes (1990) and the ORDER of USSR Ministry of Health of August 12, 1977, No 755 'On measures to further improve the organizational form of the use of experimental animals'.

Experimental protocol

Fifty-two random-bred male rats weighing 180–240 g were studied. Experiments were conducted as follows: animals with experimental MI model were divided into two groups, 15 rats in each group. The first group included white rats that received L-17 compound intraperitoneally at a dosage of 40 mg/kg every 24 h; the second group received sodium chloride solution intraperitoneally; the control group of intact animals without experimental AMI included 10 rats.

Animals were sacrificied for the study on days one, five and seven after being anesthetized with Aethaminalum-natrium intraperitoneally at a dosage of 40 mg/kg.

At harvesting of the hearts for subsequent histological evaluation, gross observations in all test cases showed the presence of the distinct infarction area in myocardium. No lung changes and no pleural effusions were noted. Gross observation did not reveal any visible changes in abdominal organs (liver, spleen, intestinal loops) or other tissues.

The myocardium from all hearts were examined sampling both areas of infarction and peri-infarction myocardial areas, as well as left ventricular areas distant from the damaged tissues. Paraffin blocks were prepared using standard methods. Serial sections (5–6 micron) of 5–6 micron were stained with haematoxylin and eosin according to Van Gieson and Weigert (Lillie 1977; Bancroft & Stevens 1996).

For biochemical analysis, 3 ml of blood was obtained by a heart puncture for subsequent centrifugation and serum separation. Serum activity of creatine phosphokinase (CPK), aspartate aminotransferase (AST), isoenzymes 1 and 2 and lactate dehydrogenase (LDH1-2) before and in the process of MI development was investigated at the same time intervals.

Laboratory blood tests were performed using the following devices and diagnostic systems:

- 'Immunochemistry Systems' biochemical analyzer by Beckman Coulter, Inc. (Brea, CA, USA)
- Diagnostic Systems by DSL, Inc. (Webster, TX, USA)
- 'Multiscan' Spectrophotometer by Labsystems Ltd. (Helsinki, Finland)
- 'Glycomat DS5' Automatic Analyzer by Drew Scientific Ltd. (Dallas, TX, USA)

Tolerability of L-17 compound

Tolerability of L-17 compound in animals was estimated as quite satisfactory: there were no detected cases of pain reactions or necrobiotic changes at injection sites, no postoperative wound infections and no pleural empyemas, except for slowing down of animals' reactions to sound and light stimulation that was noted in 20–30 min after injections and lasted 40–45 min before the animals' behaviour was not different again from that of intact rats. Symptoms of respiratory insufficiency or food and drink intake disorders were also not seen.

Statistical analysis

The differences in mean value of the various treatment groups were analysed by Scheffe's multiple comparisons in one-way analysis of variance (ANOVA). The interaction of drug treatment and treatment period was analysed by two-way ANOVA. Comparison of parametric variables was made by unpaired sample *t*-test. All the data were expressed as mean ± SD. A *P*-value <0.05 was considered statistically significant.

Results

There was no mortality of animals attributable to the procedure. There were no late deaths during the study period. At baseline and at the end of the study, there was no difference in body weight between MI and control rats.

L-17 effects on blood biochemical values in the course of experimental MI are shown in Table 1.

Table 1 Biochemical values of blood serum in the course of experimental acute MI

		Animals with experimental acute MI					
		MI, Day 1		MI, Day 5		MI, Day 7	
Values	Intact rats (n = 10)	Untreated (n = 5)	L-17 compound (n = 5)	Untreated (n = 5)	L-17 compound (n = 5)	Untreated (n = 5)	L-17 compound (n = 5)
CPK (μmol/L-min)	146.92 ± 22.6	234.9 ± 60.1*	201.08 ± 28.5*	168.54 ± 21.6*	103 ± 12.1†	248.12 ± 41.5*	107.64 ± 19.9†
AST (μmol/L-24 h)	0.193 ± 0.014	0.415 ± 0.033**	0.379 ± 0.022‡*	0.290 ± 0.05*	0.184 ± 0.011†	0.288 ± 0.023*	0.225 ± 0.024†
LDH1-2 (μmol/L-24 h)	165.15 ± 34.6	515.82 ± 60.1*	403.78 ± 38.4**	262.28 ± 22.1*	258.08 ± 31.6*	346.46 ± 52.9*	229.74 ± 60.6*

Reliability of differences between intact animals and animals with an experimental MI: *$P < 0.05$; **$P < 0.01$; reliability of differences between animals that received and did not receive L-17 compound: $^{\dagger}P < 0.05$; $^{\ddagger}P < 0.01$. AST, aspartate aminotransferase; CPK, creatine phosphokinase; MI, myocardial infarction.

Levels of enzymes

Biochemical analysis in animals with untreated experimental MI showed that the, levels of all detectable enzymes exceeded the respective values in intact animals significantly, whereas in the group treated by L-17 compound, AST and CPK levels at days 5 and 7 of experiments did not differ significantly from the values of intact animals. Besides, these levels were significantly lower compared with those of the untreated experimental MI group: for AST at days 1, 5 and 7 and for CPK for days 5 and 7. A similar trend was found for LDH1-2 values in treated and untreated experimental MI groups.

In the untreated experimental MI group, there were significantly increased CPK and LDH1-2 levels at day 7. This suggests possible the recurrence of MI that was probably induced by active motions of the animals. In the experimental MI group treated with L-17, there was no statistically significant increase in enzyme levels.

Course of experimental acute myocardial infarction

On the first postoperative day the infarction area in animals with untreated experimental MI was characterized as transmural, as evidenced by the presence of cardiomyocytes with signs of karyolysis, plasmolysis and plasmorrhexis. There was moderate diffuse infiltration of the damaged area with segmented leucocytes without formation of a demarcation zone (Figure 2). Oedema, and fullness of endomysial vessels with sludge formation was observed in the adjacent structures. Detectable polymorphonuclear leucocytes in the area of destruction were indicative of reactive inflammation with exudative response. Red blood cell sludge formation and focal haemorrhages were detected in microvessels of the perifocal area.

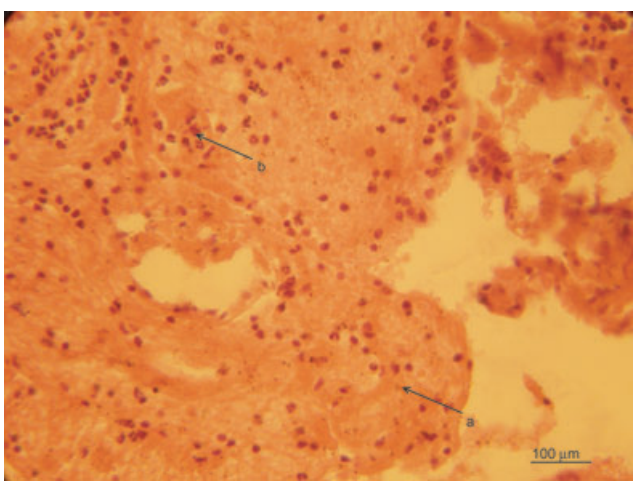

Figure 2 Myocardial infarction, day 1, without administration of the preparation. Necrosis area (a) is represented with cardiomyocytes with signs of karyolysis, plasmolysis and plasmorrhexis; moderate diffuse infiltration (b) of injured area with segmented leukocytes. H&E staining. Magnification × 400.

Significant changes were seen not only in the necrosis area but also in adjacent tissues. These changes included partial atrophy of myocardiocytes, marked dystrophic reaction with stromal oedema, and loss of cross and axial striation of myofibrils (Figure 3).

On the first postoperative day in the experimental MI animal group treated with L-17, the infarction area was large, focal and showed cardiomyocytes with signs of karyolysis, plasmolysis and plasmorrhexis without a clear demarcation zone. Small numbers of lymphocytes were present in the infiltrate. The necrotic area was minimally infiltrated (Figure 4).

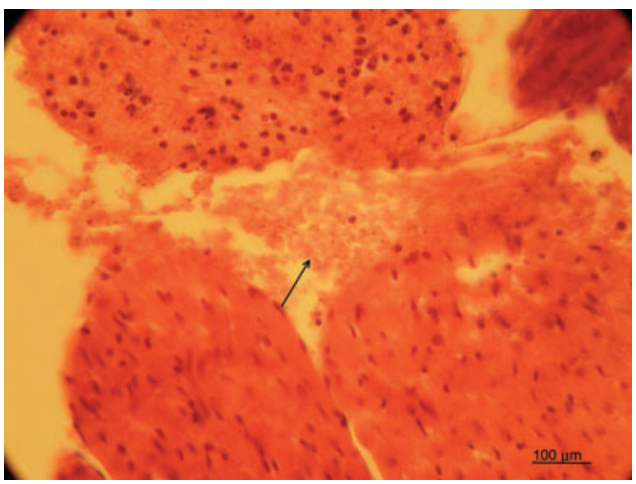

Figure 3 Myocardial infarction, day 1, without administration of the preparation. Fullness of endomysial vessels with sludge formations. H&E staining. Magnification × 400.

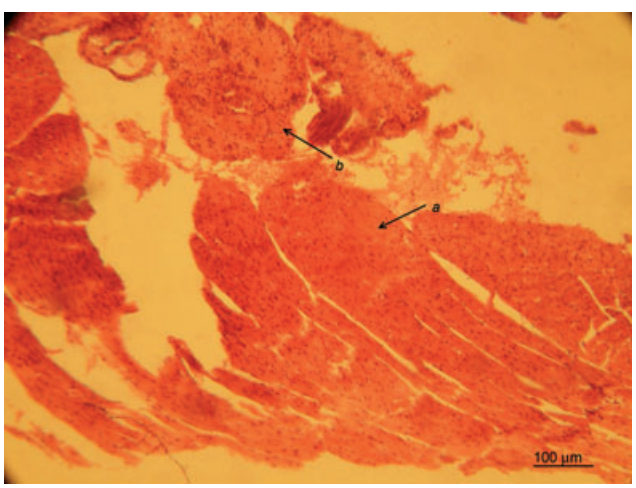

Figure 4 Myocardial infarction, day 1, with preparation administered. Damaged area (a) is represented with cardiomyocytes with signs of karyolysis, plasmolysis and plasmorrhexis; moderate diffuse infiltration (b) of the injured area is less marked; infiltrate contains small numbers of lymphocytes. H&E staining. Magnification × 400.

On the fifth postoperative day in animals with untreated experimental MI the necroyic area was characterized as predominantly transmural. Necrotic cardiomyocytes were surrounded by a demarcation line; and few signs of granulation tissue formation were observed: fibroblasts and haemocapillaries had begun to appear. Adjacent structures demonstrated infiltration of endomysium.

On the fifth postoperative day in experimental MI animals treated with L-17 compound, the injured area was replaced by granulation tissue represented by fibroblasts, fine collagen fibres and multiple sinusoidal capillaries. Granulation tissue was infiltrated with lymphocytes and macrophages, but there were few polymorphonuclear leucocytes.

On seventh postoperative day in all animals with untreated experimental MI, the necrotic area in the left ventricular wall was characterized as transmural. Histological signs of the formation stage appeared (i.e. granulation tissue formation on the edges of the necrosis area with large amount of fibroblasts, macrophages and sinusoidal haemocapillaries replacing the injured area); however, disintegration of muscular cells and infiltration of myocardium with lymphocytes and segmented leucocytes persisted. In some cases, margination with signs of leucopedesis was detected within vessels.

On seventh postoperative day in experimental MI animals treated with L-17 compound, the necrotic area was completely replaced with granulation tissue, in which multiple sinusoidal-type haemocapillaries, formation of fine collagen fibres, increased intercellular ground substance, in which significant number of functionally active fibroblasts and macrophages were detectable. The Infiltrate was composed of lymphocytes with small numbers of polymorphonuclear leucoctes. Signs of interstitial oedema persisted in the adjacent myocardial areas; endomysial vessels were dilated and full.

Discussion

The present study investigated the effects of L-17 compound of the group of substituted 5R1, 6H2-1,3,4-thiadiazine-2-amines on inflammatory cellular infiltration and myocardial remodelling after acute MI in rats.

Laboratory studies of the effects of L-17 compound on the development and course of experimental MI showed that L-17 compound administration significantly decreases the blood AST and CPK levels. Given the data about correlation between activity of these enzymes and the dimensions of the necrotic area, it allows one to draw some provisional conclusions about effectiveness in treating MI.

Histology findings in animals with experimental MI deserve special attention. L-17 compound was found not only to decrease the area of initial infarction but also to change the pattern of inflammatory reaction in the damaged myocardium fundamentally.

Basically, reduction in the amount of irreversibly damaged tissue in itself can be a major factor determining the clinical presentation of MI and its prognosis (Golikov, 2004); however, as it turned out, L-17 compound administration leads to replacement of the exudative destructive inflammation, which is mainly driven by polymorphonuclear leucocytes, with cellular productive inflammation characterized by the dominance by mononuclear cells in the infiltrate. It is probably the development of the exudative destructive inflammation that can aggravate ischaemia and reperfusion injury to the myocardium and contribute to the spread of the area of necrotic tissue (Tommasi *et al.* 1999).

Thus, the studies performed here showed that L-17 compound provides an essentially new approach to MI treatment, which means changing the pattern of inflammatory reaction in such a way as to allow the domination of protective physiological reactions of a body.

Conclusions

The use of the L-17 compound in experimental MI induces replacement of exudative destructive inflammation with cellular productive inflammation, which leads to reduction in the initial necrotic area and decrease in myocardial ischaemia and reperfusion injury and accelerates the repair process.

Acknowledgements

This study was supported by a Special Research Grant of Interdisciplinary project of the Institute of Immunology and Physiology and the Institute of Organic Synthesis, Ural Branch of RAS (2009–2011).

References

Arzamastsev A.P., Severina I.S., Grigoryev N.B., Granik V.G. (2003) Exogenic donors of nitric oxide and inhibitors of NO-sintases (chemical aspect). *Vestn Ross Akad Med Nauk* **12**, 88–95.

Bancroft J.D. & Stevens A. (eds) (1996) *Theory and Practice of Histological Techniques*, 4th edn. New York, NY: Churchill Livingstone.

Chukaeva I.I., Bogova O.T., Korochkin I.M. (2007) Myocardial infarction and inflammation. *Emerg. Med.* **4**, 19–23.

Dinerman J.L., Mehta J., Saldeen T.G. *et al.* (1990) Increased neutrophil elastase release in unstable angina pectoris and acute myocardial infarction. *J. Am. Coll. Cardiol.* **7**, 1559–1563.

Drapkina O.M., Zadorozhnaya O.O., Ivashkin Drapkina V.T. (2000) Characteristics of nitric oxide synthesis in myocardial infarction patients. *Clin. Med.* **3**, 19–23.

Engler R.L., Dahlgren M.D., Morris D.D. *et al.* (1986) Role of leukocytes in response to acute myocardial ischemia and reflow in dogs. *Am. J. Physiol.* **251**, 314–332.

Golikov P.P. (2004a) *Nitric Oxide in Clinical Emergency*. Moscow: Medpraktika.

Hudson M.P., Christenson R.H., Newby L.K. (1999) Cardiac markers: point of care testing. *Clin. Chim. Acta* **284**, 223–237.

Kuzuya T., Fuji H., Hoshida S. *et al.* (1991) Neutrophil-induced myocardial cell damage and active oxygen metabolites. *Jpn. Circ. J.* **11**, 1127–1131.

Lillie R.D. (1977) *H.J. Conn's Biological Stains*, 9th edn. Baltimore, MD: Williams and Wilkins.

Logvinova Y.S., Vasil'eva T.M., Makarov V.A., Chupakhin O.N., Sidorova L.P., N.M., Rusinov V.L. (2010) The effect of new compounds 1,3,4-thiadiazine class on platelet aggregation in vitro and ex vivo. *Exp. Clin. Pharmacol.* **8**, 21–26.

Meyerson F.Z. (1984) *Pathogenesis and Prevention of Stressor and Ischemic Heart Injuries*. Moscow: Medicine.

Myagkov I.I., Trotsyuk V.R., Yasnitskaya M.Y. (1993) Kinin modulation therapy in acute myocardial infarction patients. *Cardiologiia* **1**, 41–43.

Novikova A.P., Perova N.M., Chupakhin O.N. (1992) Synthesis and properties of functional derivatives of 1,3,4-thiadiazines and condensed systems based on these compounds. *Chem. Heterocyclic Comp.* **11**, 1443–1457.

Saprunova V.B., Bakeeva L.E., Yaguzhinskiy L.S. (2003) Ultrastructure of mitochondrial mechanism of apoptotic rat cardiomyocytes induced by prolonged anoxia. *Cytology* **11**, 1074–1082.

Sarkisov D.S. (1979) *Regeneration and its Clinical Significance*. Moscow: Medicine.

Smith S.J., Bos G., Essvild R. (1977) Acute – phase proteins from the liver and enzymes from myocardial infarction, a quantitative relationship. *Clin. Chem. Acta* **81**, 75–85.

Tommasi S., Carluccio E., Bentivoglio M. *et al.* (1999) C-reactive protein as a marker for cardiac ischemic events in the year after a first, uncomplicated myocardial infarction. *Am. J. Cardiol.* **12**, 1595–1599.